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Data Sheet

PRMT5 Chemiluminescent Assay Kit

Catalog # 52002L

DESCRIPTION: The *PRMT5 Chemiluminescent Assay Kit* is designed to measure PRMT5 activity for screening and profiling applications. The *PRMT5 Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well plate precoated with histone H4 peptide substrate, the antibody against methylated arginine3 residue of Histone H4, the secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified PRMT5 enzyme for 100 enzyme reactions. The key to the *PRMT5 Chemiluminescent Assay Kit* is a highly specific antibody that recognizes methylated R3 residue of Histone H4. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme for one hour. Next, primary antibody is added. Finally, the plate is treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Components	Amount	Storage	
51045	PRMT5/MEP50 human enzyme	20 µg	-80°C	***Avoid freeze/thaw cycles!***
52120	S-adenosylmethionine	250 µl	-80°C	
52150	Primary antibody 4	100 µl	-80°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
52170	4x HMT assay buffer 2	3 ml	-20°C	
52100	Blocking buffer	50 ml	+4°C	
	HRP chemiluminescent substrate (2 components)	6 ml each	+4°C	
	Black plate precoated with histone substrate	1	+4°C	

MATERIALS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1 x TBS, pH 8.0, containing 0.05% Tween20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt

STABILITY: One year from date of receipt when stored as directed.

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REFERENCE: Dillon SC, Zhang X, Trievel RC, Cheng X. *Genome Biology* 2005; **6:**227.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 150 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the plate onto clean paper towels to remove liquid.

2) Thaw S-adenosylmethionine on ice. Upon first thaw, briefly spin tube containing S-adenosylmethionine to recover full content of the tube. Aliquot S-adenosylmethionine into single use aliquots. Store remaining S-adenosylmethionine in aliquots at -80°C immediately. Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.

3) Prepare the master mixture: N wells × (7.5 µl **4× HMT assay buffer 2** + 2.5 µl **400uM S-adenosylmethionine** + 15 µl **H_2O**)

4) Thaw **PRMT5/MEP50 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT5/MEP50 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. Note: **PRMT5/MEP50 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

5) Dilute **PRMT5/MEP50 enzyme** in 1× HMT assay buffer 2 at 10 ng/µl (200ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

Add 25 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the "Substrate Control", add 7.5 µl **4× HMT assay buffer 2** + 17.5 µl **H_2O**

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	Blank	Substrate Control	Positive Control	Test Inhibitor
4× HMT assay buffer 2	7.5 µl	7.5 µl	7.5 µl	7.5 µl
400 µM S-adenosylmethionine	2.5 µl	–	2.5 µl	2.5 µl
H_2O	15 µl	17.5 µl	15 µl	15 µl
Test Inhibitor/Activator	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1× HMT assay buffer 2	20 µl	–	–	–
PRMT5/MEP50 (10 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

6) Add 5 µl of inhibitor solution of each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer)

7) Add 20 µl of 1 × HMT buffer 2 to the well designated "Blank".

8) Initiate reaction by adding 20 µl of diluted PRMT5/MEP50 prepared as described above. Incubate at room temperature for 1 hour.

9) Wash the plate three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

10) Add 100 µl of Blocking buffer to every well. Shake on a rotating platform for 10 min. Remove supernatant as above.

Step 2:

1) Dilute **"Primary antibody 4"** 100-fold with Blocking buffer.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash plate three times with 200 µl TBST buffer and incubate in Blocking buffer as in steps 1-9 and 1-10.

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Step 3:

1) Dilute "**Secondary HRP-labeled antibody 2**" 1,000-fold with Blocking buffer.

2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash plate three times with TBST buffer and incubate in Blocking buffer as in steps 1-9 and 1-10.

4) Just before use, mix on ice 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

5) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavenlength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

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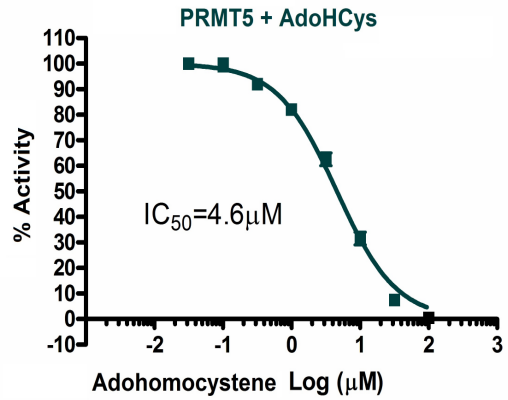
Example of Assay Results:



PRMT5 enzyme activity, measured using the *PRMT5 Chemiluminescent Assay Kit*, BPS Bioscience Catalog #52002L. Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS

PRMT5/MEP50 (expressed in Sf9 cells)	#51048	20 µg
PRMT1 (expressed in E. coli)	#51040	50 µg
PRMT1 (expressed in Sf9 cells)	#51041	20 µg
PRMT3 (expressed in E. coli)	#51043	50 µg
PRMT4/CARM1 (expressed in HEK293)	#51047	20 µg
PRMT5/MEP50 (expressed in HEK293)	#51045	20 µg
PRMT6 (expressed in HEK293)	#51049	20 µg
PRMT7 (expressed in Sf9)	#51054	20 µg
PRMT8 (expressed in Sf9)	#51052	20 µg
PRMT9 (expressed in Sf9)	#51053	20 µg
PRMT1 Chemiluminescent Assay Kit	#52004L	96 reactions
PRMT3 Chemiluminescent Assay Kit	#52005L	96 reactions
PRMT4 Chemiluminescent Assay Kit	#52041L	96 reactions
PRMT6 Chemiluminescent Assay Kit	#52046	96 reactions
PRMT1 Homogeneous Assay Kit	#52054	384 reactions
PRMT3 Homogeneous Assay Kit	#52055	384 reactions
PRMT5 Homogeneous Assay Kit	#52052	384 reactions
PRMT6 Homogeneous Assay Kit	#52056	384 reactions
PRMT8 Homogeneous Assay Kit	#52058	384 reactions

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	PRMT5 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (PRMT5, BPS Bioscience #51045). Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Record light signals at 5 second intervals. Refer to instrument instructions for settings to increase sensitivity of light detection. See "Reading Chemiluminescence" section.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of enzyme (PRMT5, BPS Bioscience #51045) to create a standard curve.

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